INVENTING FOR LIFE

Enabling Technologies for Organic Chemistry (ETOC) Symposium

February 24-25, 2022

@AlexandraCSun

Vision-Guided, High-Throughput Liquid-Liquid Extraction Screening

presented by

Alexandra Sun

Enabling Technologies,
Process R&D, Merck & Co., Inc., Kenilworth, NJ, USA

Data-Rich Experimentation (DRE) Group

Shane Grosser
Group lead, Technology enthusiast

Alex Sun
HTE, Automation

Melodie Christensen
HTE, Automation

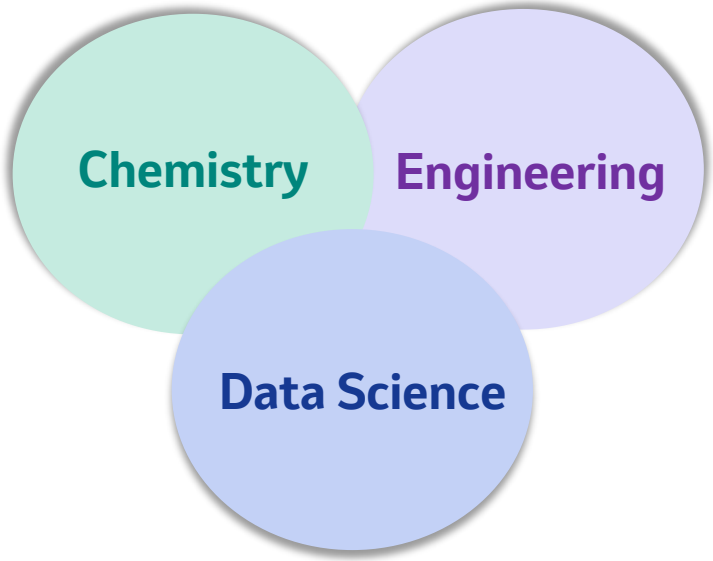
Daniel Holland-Moritz
HTE, Microfluidics

Eugene Kwan
Data Science, Mechanistic Analysis

Harrison Rose
Process Modeling, Data Analysis

Keith Mattern
Custom reaction system design and integration

Kevin Stone
Process Modeling, Data Science



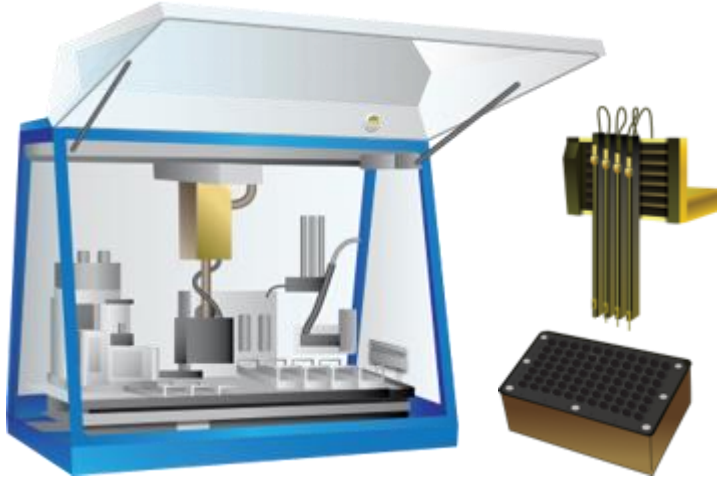
Ajit Vikram
Data Science, ML

Ivan Skvortsov
Automation, Data Analytics

Data-Rich Experimentation (DRE) Group

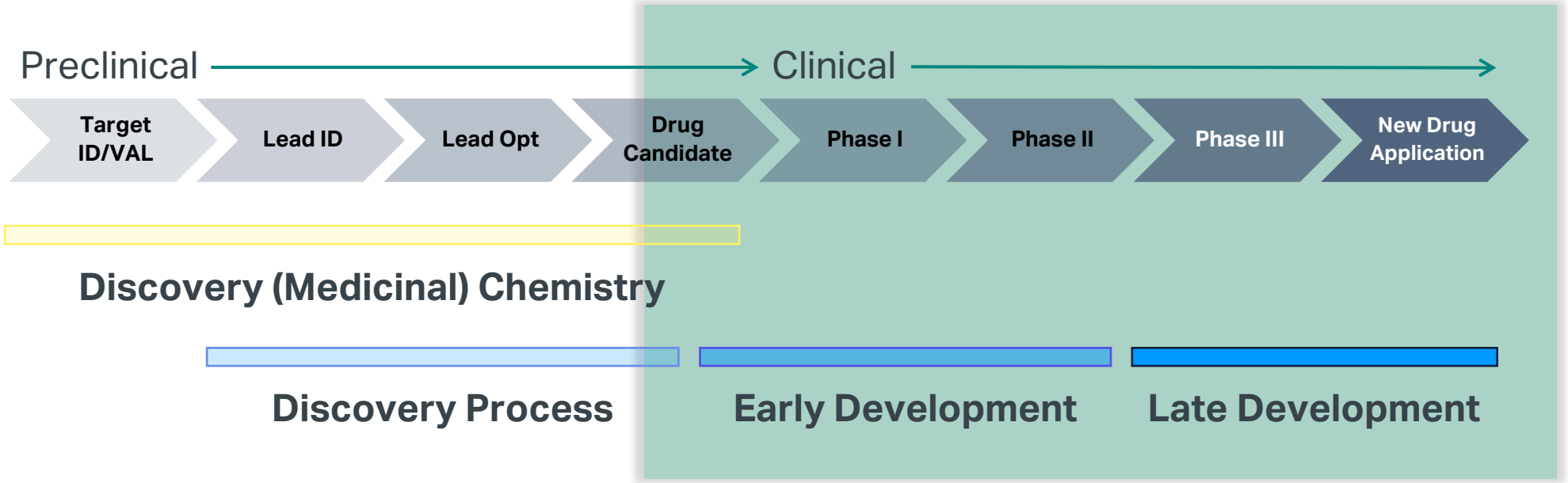
Our mission is ***to develop and deploy cutting edge, high data-density solutions to maximize the knowledge generated from every experiment*** *conducted across PR&D.*

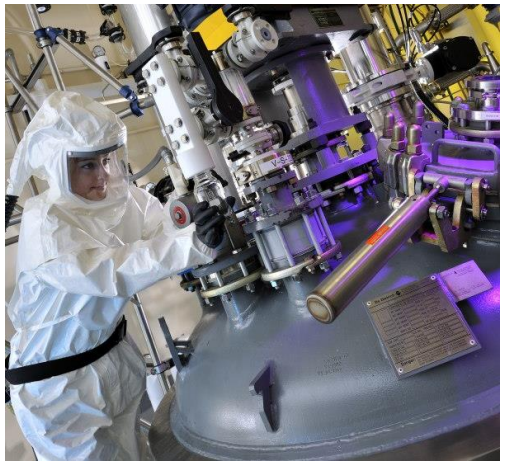
Reaction Discovery	Process Understanding	Process Optimization



Throughput → **Scale**

Leveraging DRE for small molecule process development



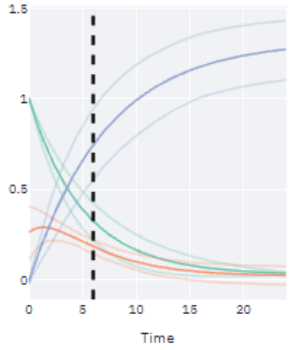


Leveraging DRE for small molecule process development

Reaction

High-Throughput Reaction Screening

Process Analytical Technologies

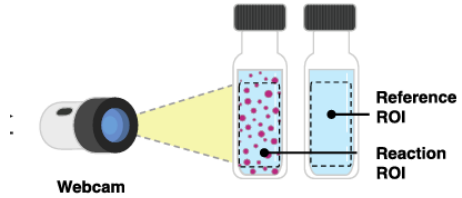


Predictive Process Understanding

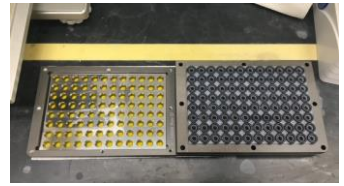
Workup

?

Crystallization



Automated Solubility Screening

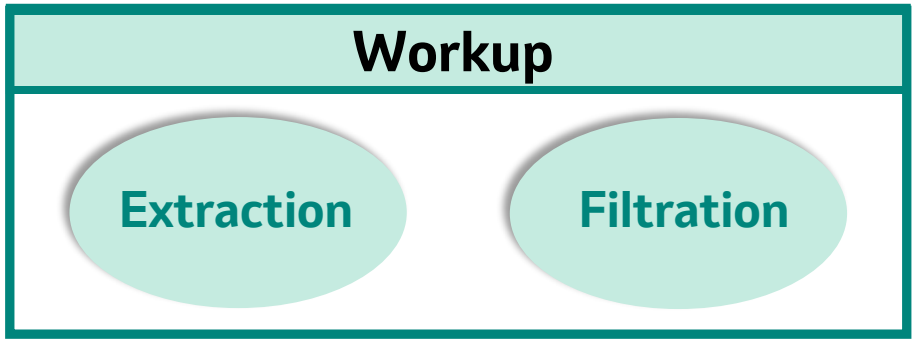


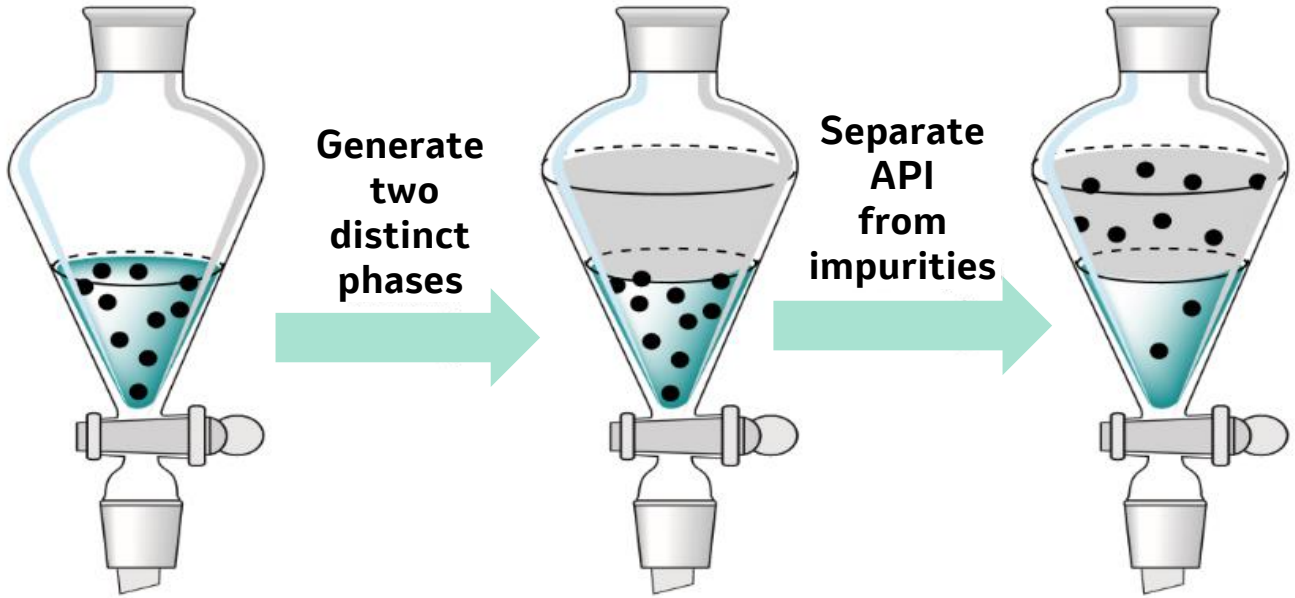
High-Throughput Powder X-ray Diffraction

How can we use DRE to develop more robust and sustainable workup processes?

iScience, **2021**, *24*, 3.

Liquid-Liquid Extraction (LLE) as a workup strategy





Benefits of Liquid-Liquid Extractions:

1. Isolation of API from hydrophilic impurities
2. Robust to changes to reaction conditions
3. Scale-up is thermodynamically controlled and not equipment or scale-dependent

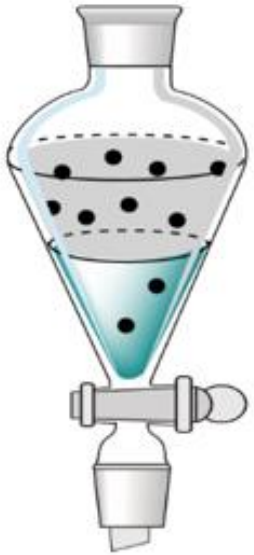
Current Limitations:

1. Timelines and development priorities
2. Incomplete stability understanding
3. **Complexity of optimization**

Optimizing conditions for LLE

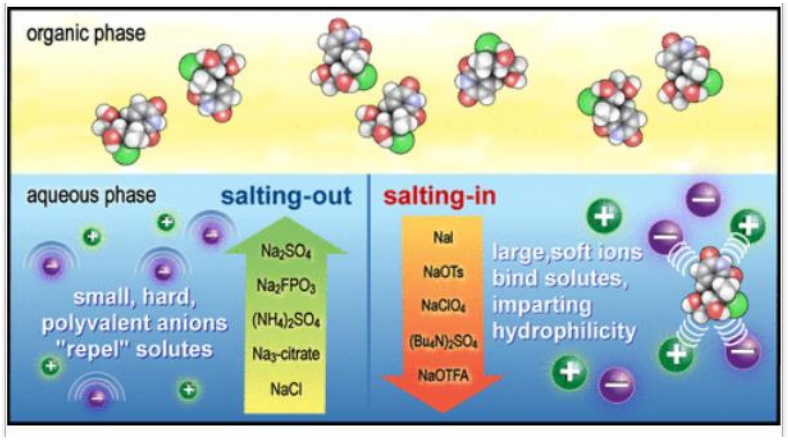
Screening Parameters (Input)

- ✓ Organic Solvents
- ✓ Organic/Aqueous Phase Ratios
- ✓ Temperature, pH
- ✓ Salts and Additives



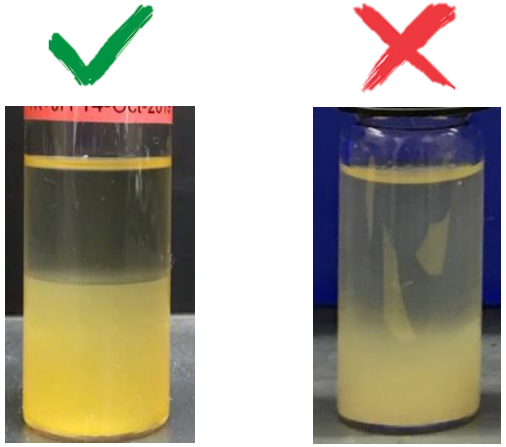
Analysis Parameters (Output)

- ✓ Distribution coefficient
- ✓ **Interface quality**
- ✓ **Phase ratio**



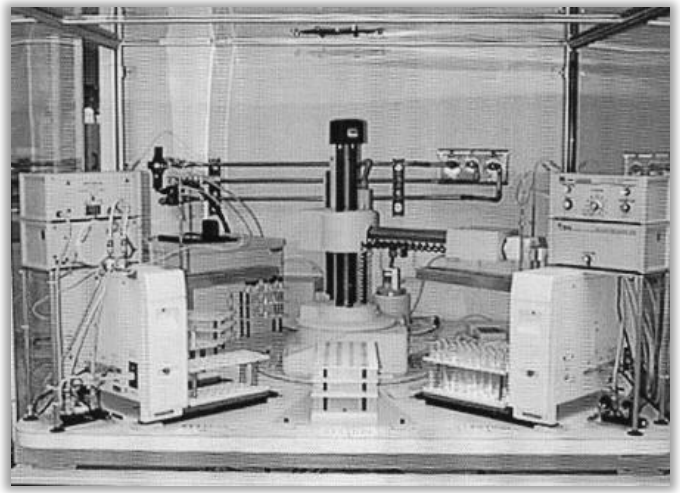
Salting-out strategies informed by the Hofmeister series

Org. Process Res. Dev. **2017**, *21*, 1355-1370



HTE platforms for LLE

LLE Robot (Abbot, 2000)



Interface detection using refractometer flow cell

80 samples per screen (15 mL)

High-Throughput LLE (BMS, 2016)

Visual analysis performed manually using visualization plate

24 samples per screen (2-4 mL)

Automated LLE Screening (GSK, 2021)

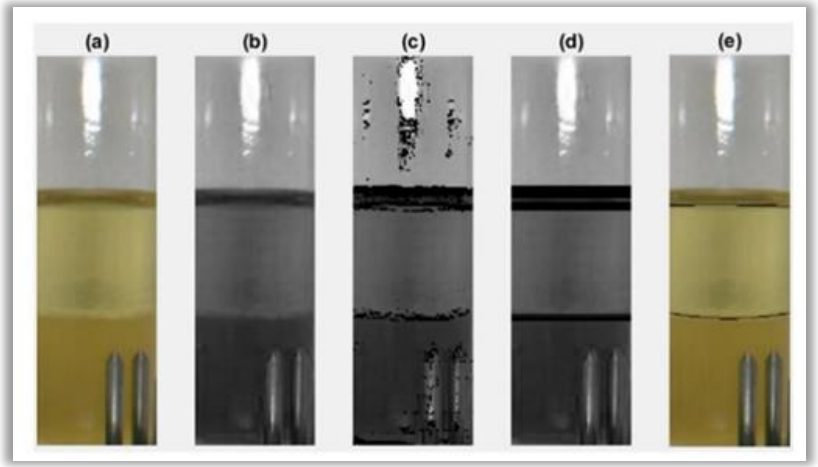


Image analysis algorithm enables automated visual analysis

24 samples per screen (2-4 mL)

JAMMC, **2000**, *22*, 187-194.; *Org. Process. Res. Dev.* **2016**, *20*, 1728-1737.; *Org. Process. Res. Dev.* **2021**, *25*, 2738-2746.

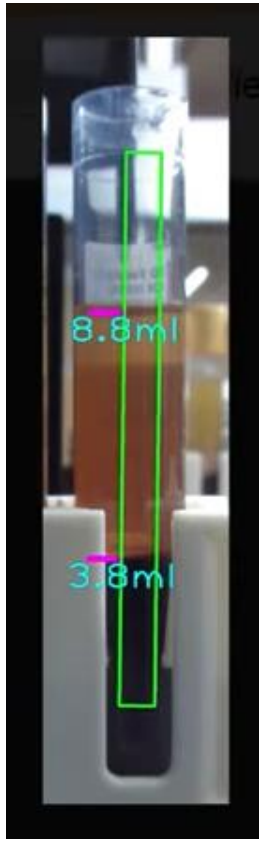
How can we increase screening throughput?

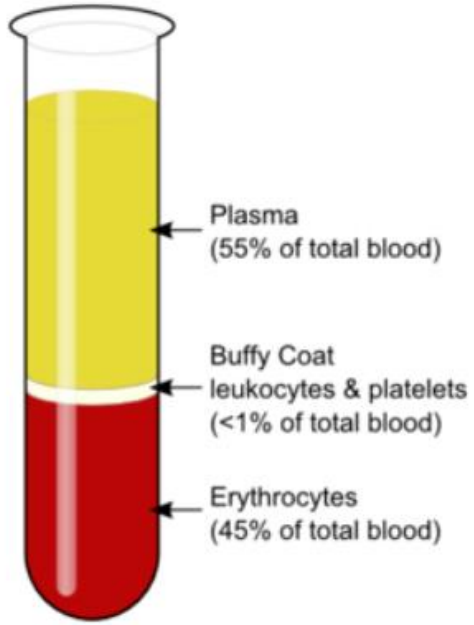
<u>This work:</u>
Automated LLE Screening using the Tecan Platform

Automated image analysis using TubeEyeX camera

96 samples per screen (0.5 – 1 mL)

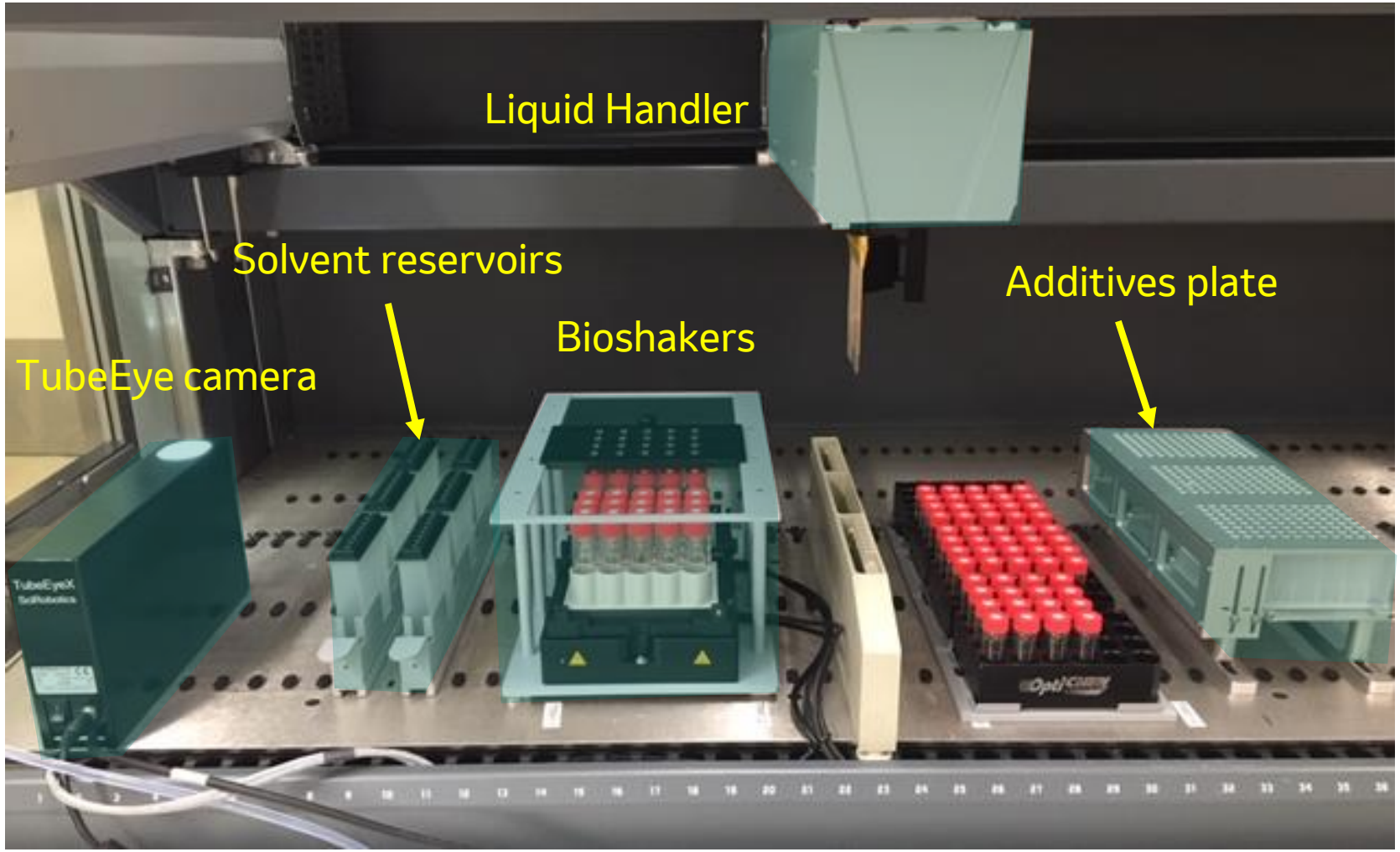
TubeEyeX camera:
Automated buffy coat extractions





Automated LLE screening workflow

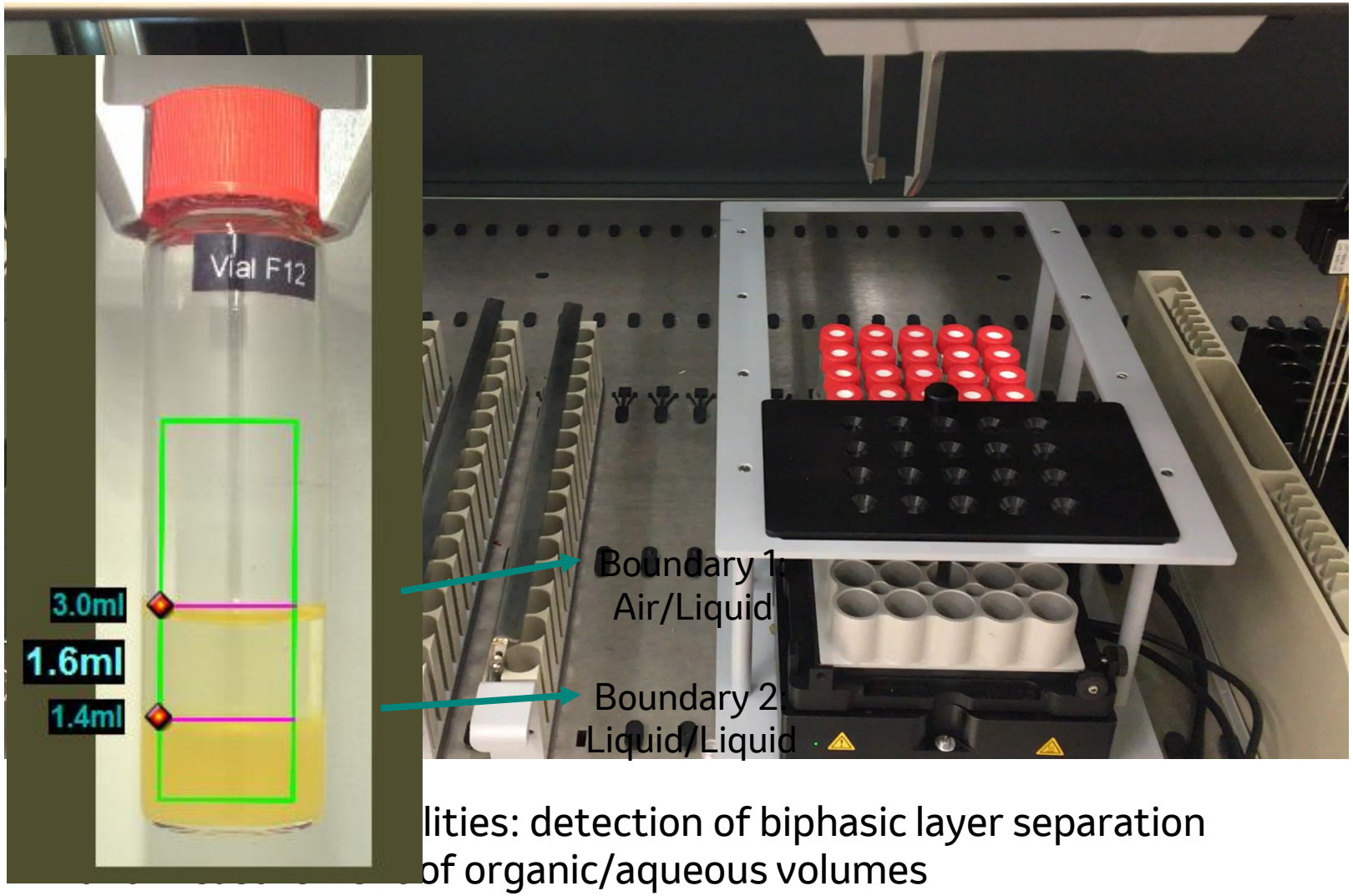
- Sample preparation
- Agitation and settling
- Image capturing and hit identification
- Organic and aqueous layer sampling
- Data processing and visualization



Throughput: 96 samples (~50 mL EOR) per run, 4-5 h run time

LLE screening automation workflow

- Sample preparation
- Agitation and settling
- Image capturing and hit identification
- Organic and aqueous layer sampling
- Data processing and visualization



lities: detection of biphasic layer separation
of organic/aqueous volumes

LLE screening automation workflow

- Sample preparation
- Agitation and settling
- Image capturing and hit identification
- Organic and aqueous layer sampling
- Data processing and visualization

LLE screening automation workflow

Sample preparation

↓

Agitation and settling

↓

Image capturing and hit identification

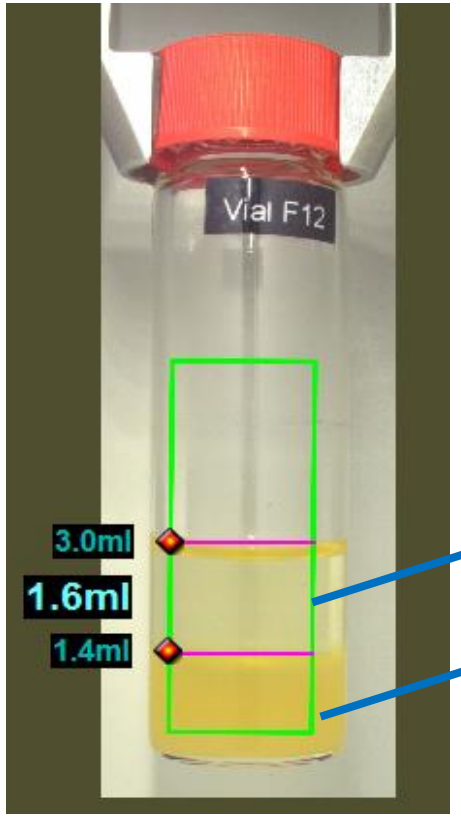
↓

Organic and aqueous layer sampling

↓

Data processing and visualization

Phase Height → ***Sampling Height***

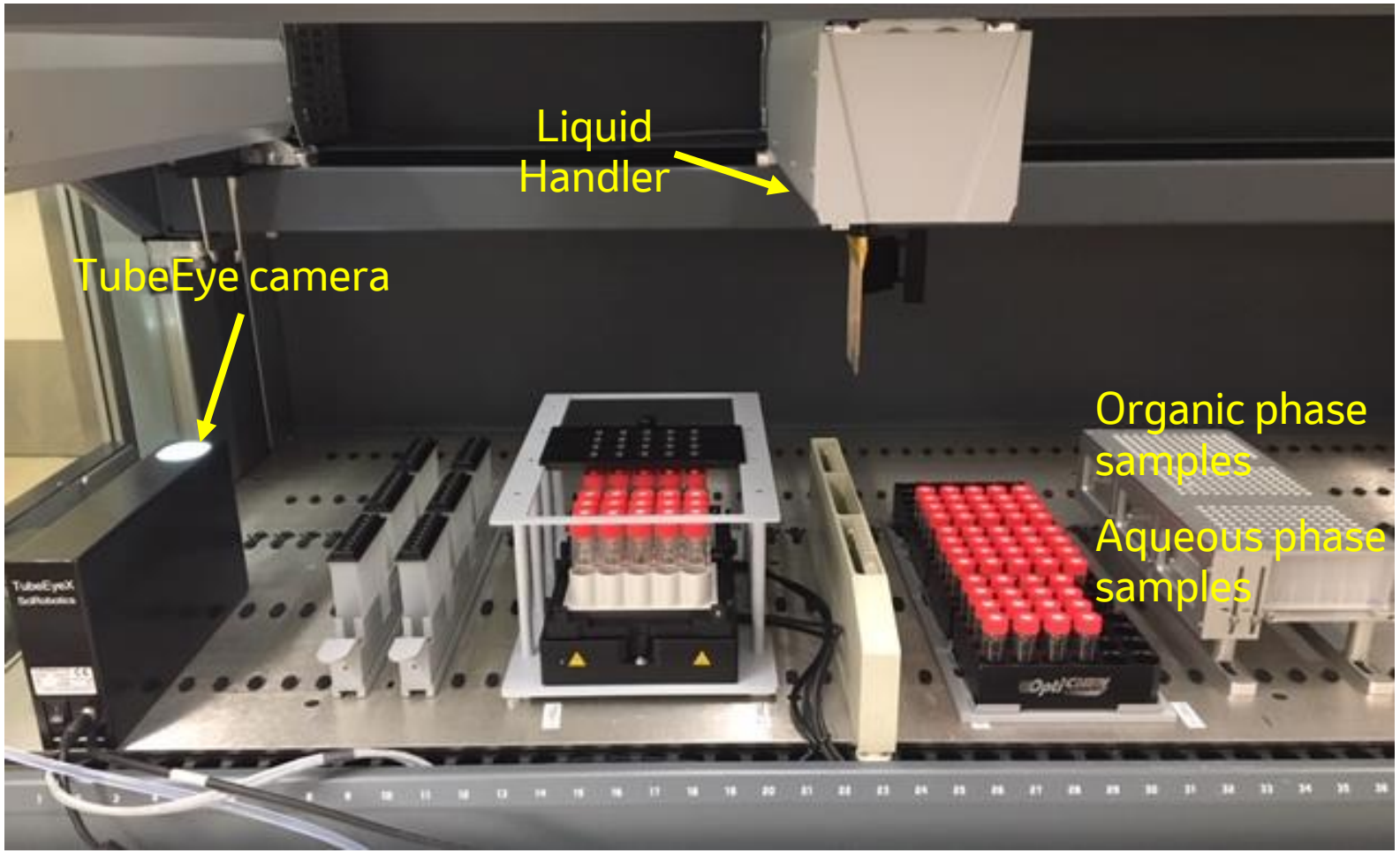


Organic sampling level:
Middle of Org. Phase

Aqueous sampling level:
Lowest Z height

LLE screening automation workflow

- Sample preparation
- Agitation and settling
- Image capturing and hit identification
- Organic and aqueous layer sampling
- Data processing and visualization



- Automated preparation of organic and aqueous phase LC samples

LLE screening automation workflow – automated data analysis

Sample preparation

↓

Agitation and settling

↓

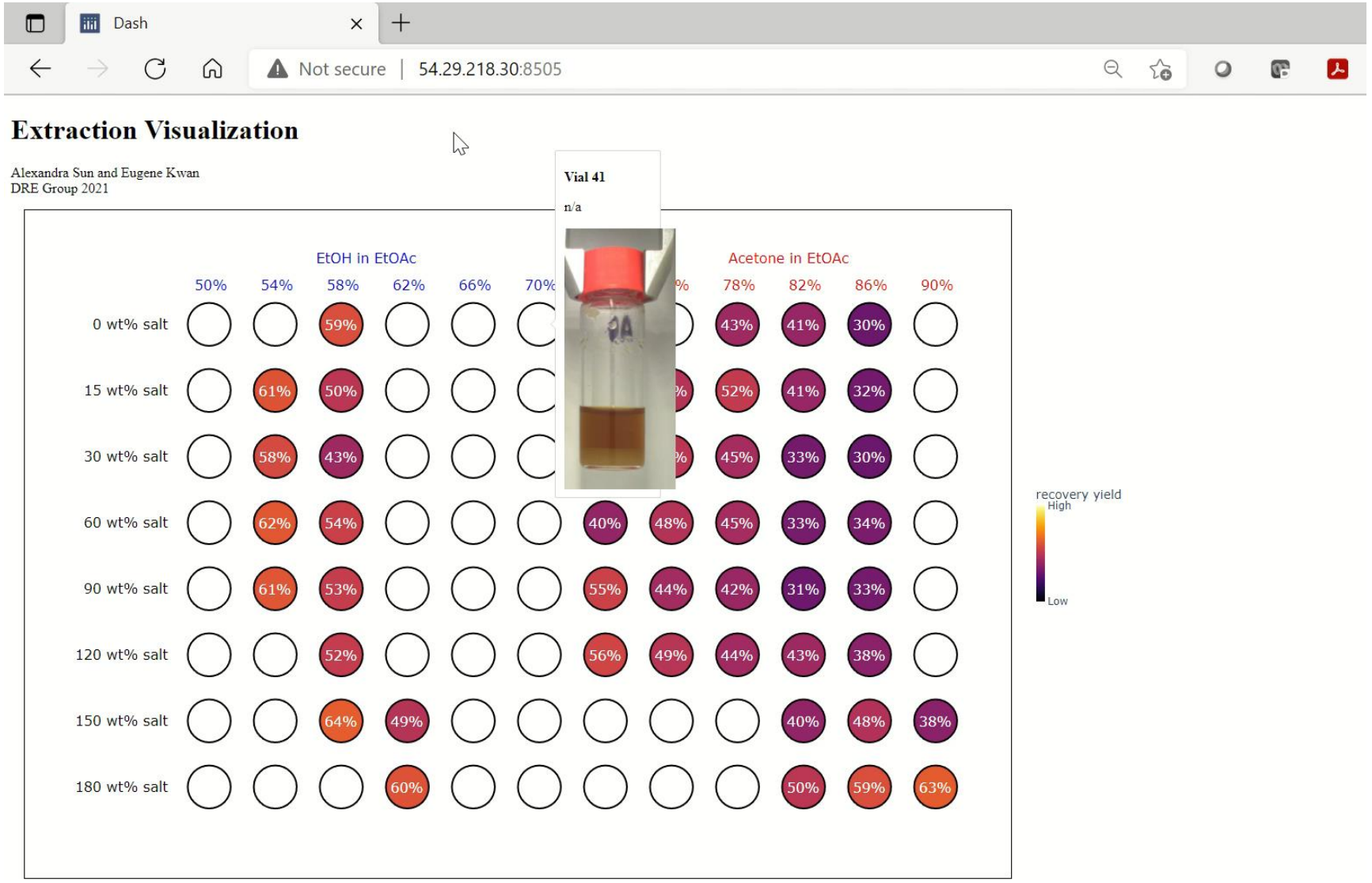
Image capturing and hit identification

↓

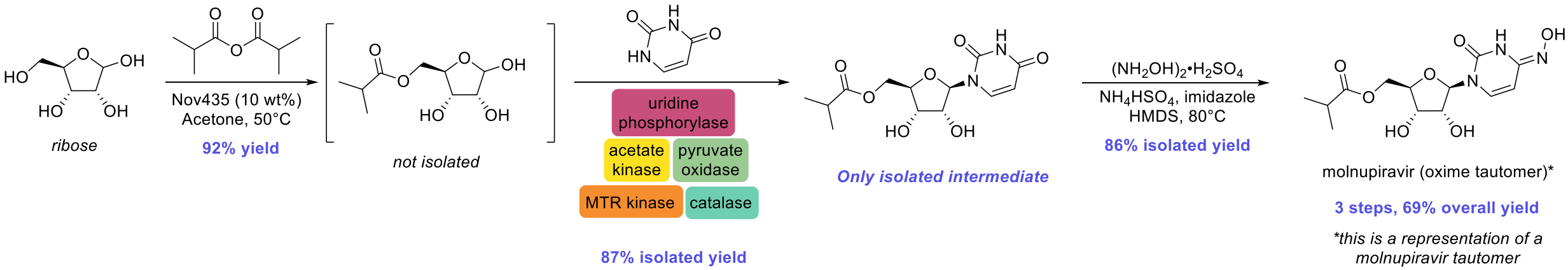
Organic and aqueous layer sampling

↓

Data processing and visualization



Case Study: Biocatalytic synthesis of Molnupiravir



Innovative chemistry enables a 3-step route to molnupiravir

Only readily-available commodity reagents and enzymes

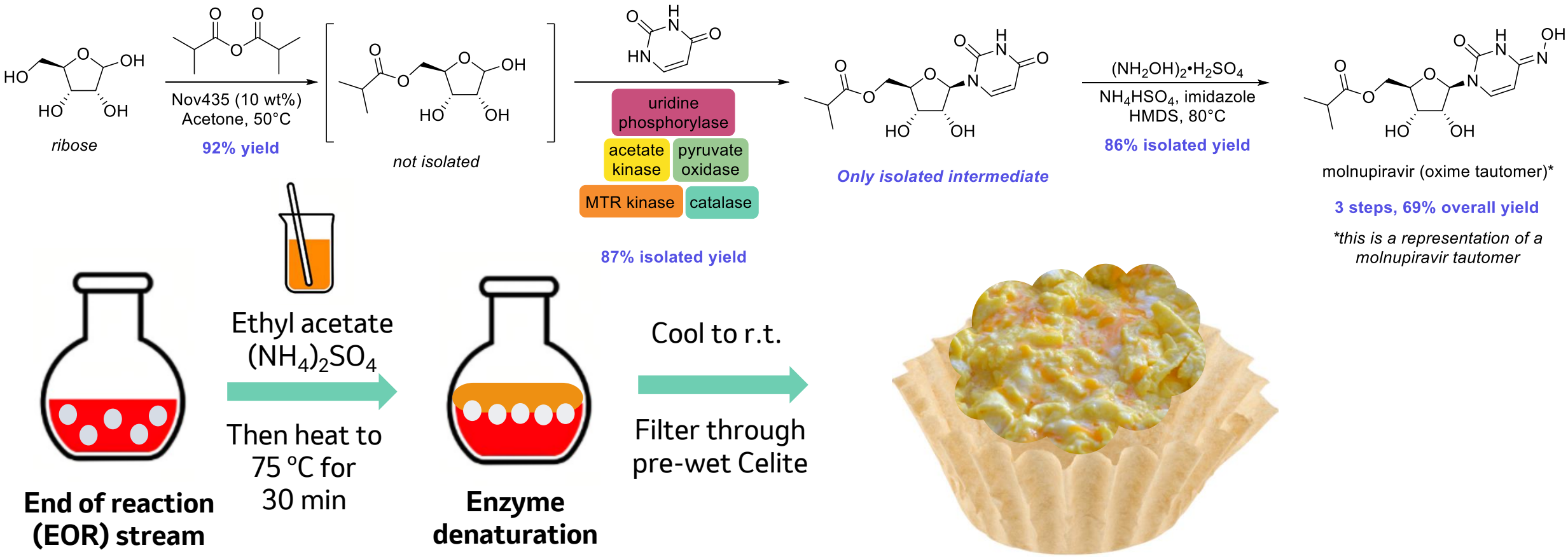
67% fewer steps and 2.5-fold higher yielding route to molnupiravir from ribose and uracil

Approximately 50% cost of goods reduction from 1st route

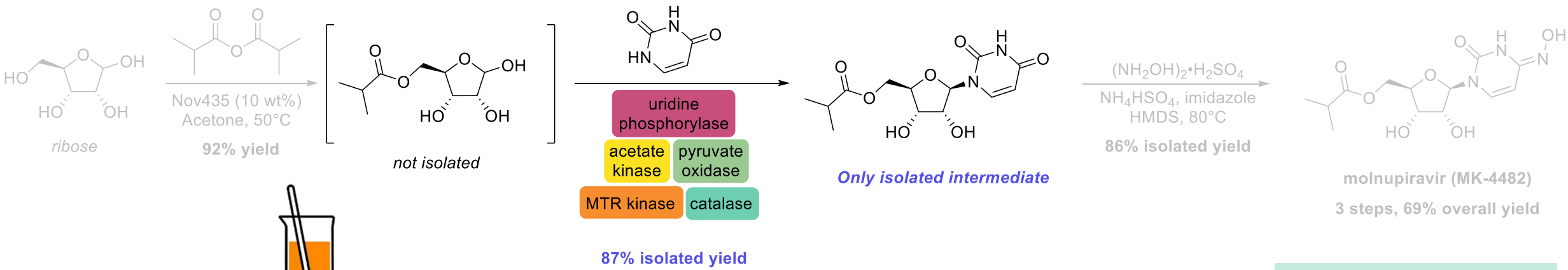
Developed and piloted in 6 months – demonstrates the speed at which enzymatic processes can be implemented to manufacture APIs

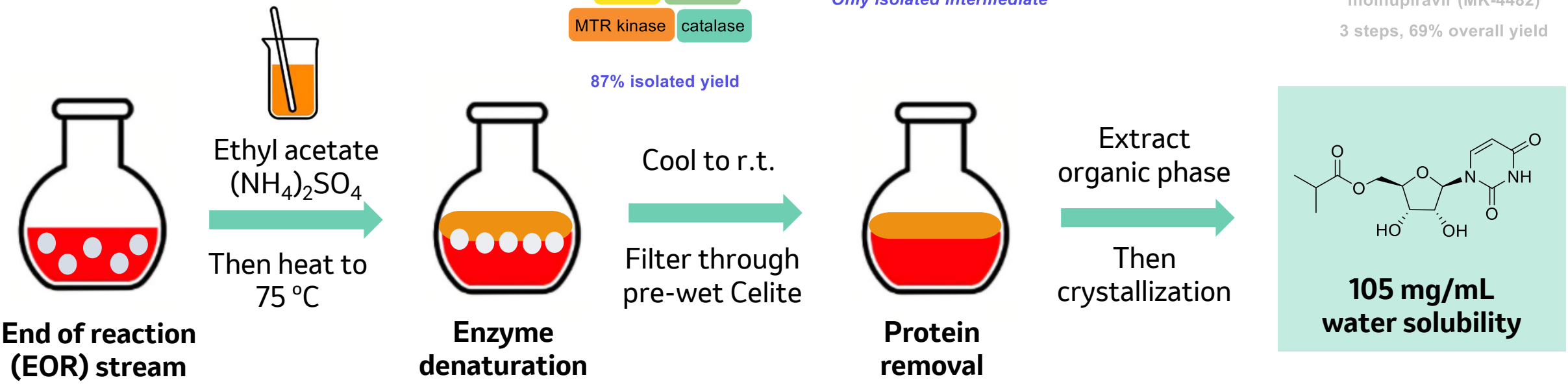
ACS Cent. Sci. **2021**, *7*, 1980-1985.

How do we remove enzyme at the end of a biocatalytic reaction?



How do we remove enzyme at the end of a biocatalytic reaction?

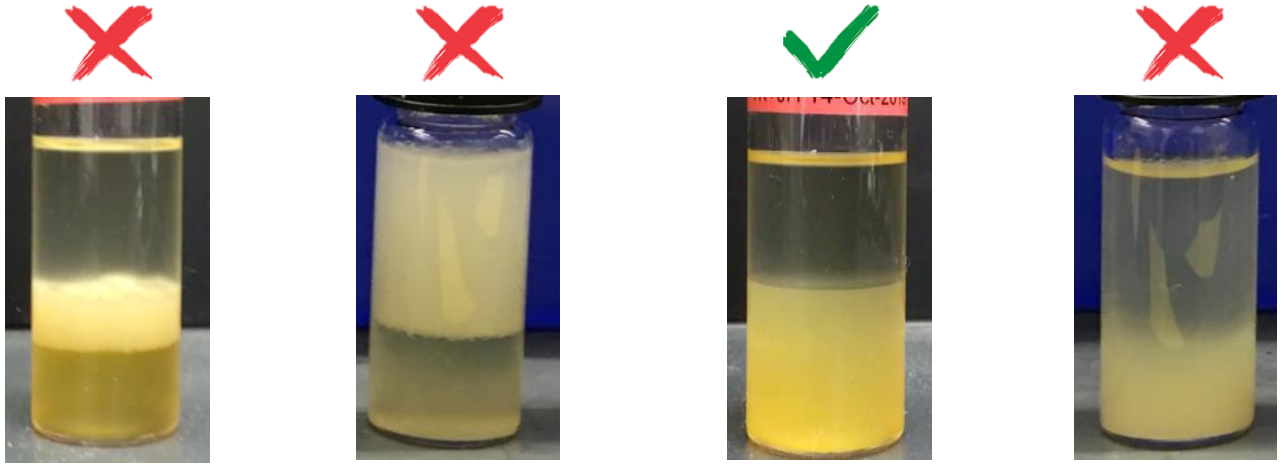




Challenge: High water solubility of 5'-isobutyryl uridine ***and*** enzyme rag layer formation prevents development of a direct extraction strategy

Developing an LLE strategy for protein removal

Objective: Identify direct extraction conditions for **enzyme removal** and **>80% recovery** of 5'-isobutyryl uridine **after a single extraction**



Protein rag | Cloudy organic | Clean Interface | Miscible

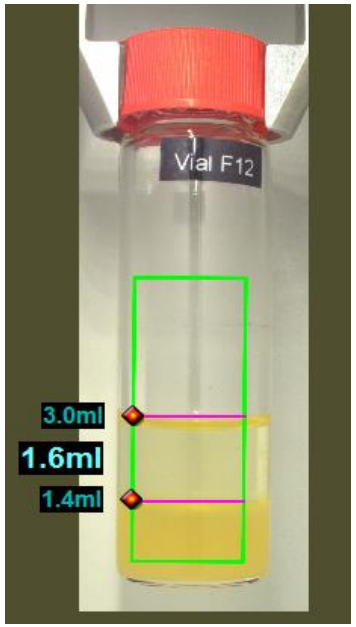
Criteria 1: Induce clean layer separation

Criteria 2: High extraction efficiency

LLE Screening strategy: Additive libraries

Hampton Research Additive Plates

Additives classes screened:
- Inorganic salts
- Amino acids
- Dissociating agents
- Linkers
- Polymers
- Polyamines/chelating agents
- Carbohydrates
- Detergents
- Organic solvents
- And many more….



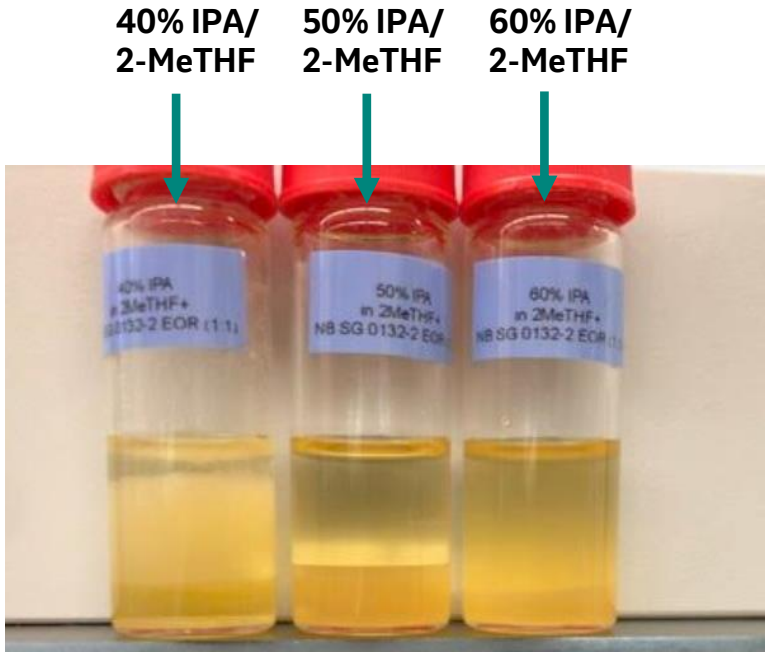
- Only **one PPG400 additive** yielded layer separation
- Extraction efficiency of ~60% required further optimization

LLE Screening strategy: Organic solvents

2-MeTHF/Alcohol co-solvent

0% alcohol → 100% alcohol

t-amylOH										
iprOH						71%				
iBuOH										
nBuOH										



- Most co-solvents provided inseparable layers
- **Only 50% IPA/2-MeTHF** yielded a phase split with 71% recovery

LLE Screening strategy: Inorganic salts

Sodium Sulfate **Ammonium Sulfate**

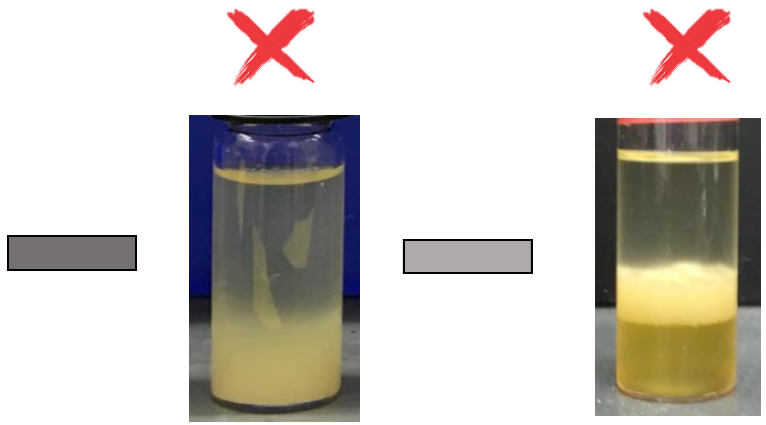
40% 2-MeTHF/IPA **60% 2-MeTHF/IPA**

wt% salt relative to substrate

0 **300**

	1	2	3	4	5	6	7	8	9	10	11	12
A			73%						69%			
B												
C							60%	45%		45%		
D	60%						70%	79%				
E												
F												
G												
H												

*Acyl Uridine extraction yield expressed as % in Organic Layer.



Miscible Protein rag

LLE Screening strategy: Inorganic salts

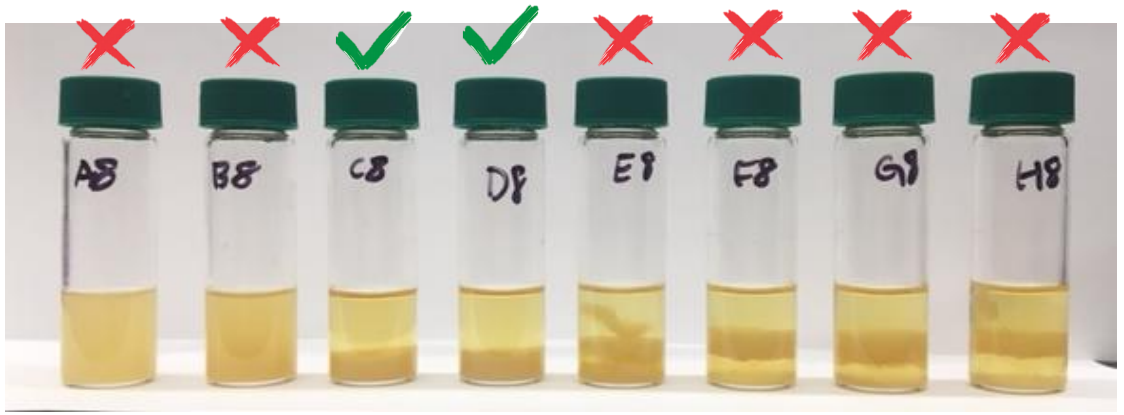
Sodium Sulfate | **Ammonium Sulfate**

40% 2-MeTHF/IPA → **60% 2-MeTHF/IPA**

wt% salt relative to substrate: **0** → **300**

	1	2	3	4	5	6	7	8	9	10	11	12
A			73%						69%			
B												
C							60%	45%		45%		
D	60%						70%	79%				
E												
F												
G												
H												

*Acyl Uridine extraction yield expressed as % in Organic Layer.



0 **15** **30** **60** **120** **180** **240** **300**

Ammonium sulfate concentration (wt%)

HTE-Enabled LLE optimization

Additives
- PPG 400
- 60% recovery

Solvents
- IPA/MeTHF
- 70% recovery

Salts
- 60 wt% $(NH_4)_2SO_4$
- 80% recovery

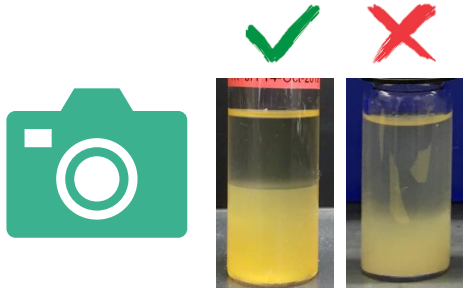
Product recovery: 90% after single extraction on 50 mL scale

Final Extraction Conditions:
44% 2-MeTHF/IPA,
60 wt% ammonium sulfate

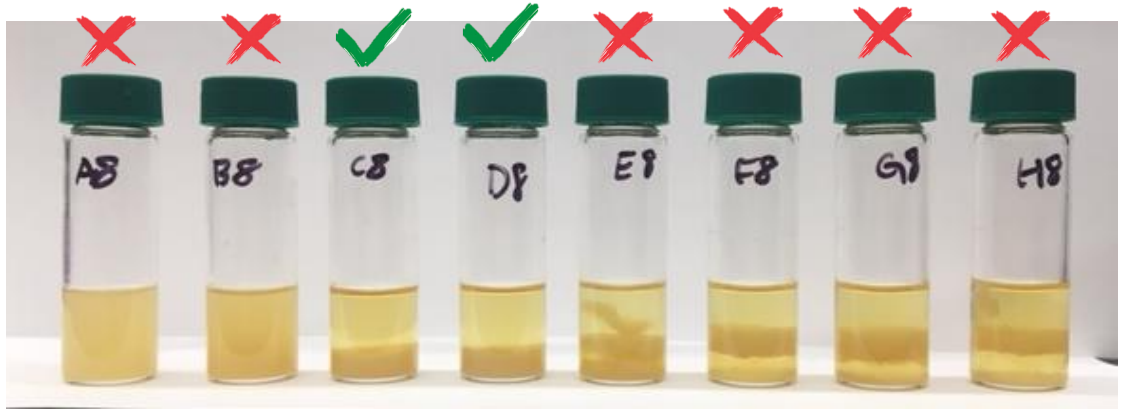
>700 conditions evaluated
only 100 of these samples gave a phase split!

Summary

- Development of a vision-guided HTE LLE platform



- Successful pipeline application enables enzyme removal



- New technology can be implemented when pre-investment and commitment to innovation exist

- Cross-disciplinary collaboration enables development of new DRE capabilities for workup optimization

Acknowledgements

Tecan Development

Jon Jurica

Shane Grosser

Eugene Kwan

Melodie Christensen

Amani Shaikh (Tecan)

Molnupiravir Project Team

Gilmar Brito

Patrick Fier

Tetsuji Itoh

Umme Ayesa